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Pathogenicity of Fusarium spp.

to Lodgepole Pine

1985

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ABSTRACT

Pathogenicity tests of ten Fusarium isolates from Colorado and Nebraska nursery soils and conifer seedlings against lodgepole pine seed and seedlings were conducted. To test for damping-off, each Fusarium isolate was tested against 50 germinating seeds with 5 seeds equidistant from a 6 mm plug of Fusarium mycelium per plate of moist filter paper. Fifty seeds were germinated uninoculated as checks. To test for cortical rot, each Fusarium isolate was tested against 50 seedlings growing in containers in a growth chamber. Each container was inoculated with a 6 mm plug of Fusarium mycelium covered with moist cotton. Seedlings were stressed for 2 months before the experiment was terminated. Unhealthy seeds and seedlings were plated on medium selective for Fusarium to determine whether the seedlings were infected with the same Fusarium with which they were inoculated. Up to 76% of the lodgepole pine seed was destroyed by damping-off due to Fusarium infection. Although none of the container seedlings showed the cortical sloughing typical of Fusarium root rot, a high percentage of the withered and chlorotic seedlings were infected with the same Fusarium with which they were inoculated. The most pathogenic Fusarium species were F. moniliforme, F. oxysporum, F. solani, and F. acuminatum.

INTRODUCTION

Many different species of Fusarium are commonly found in soil, but not all are plant pathogens. In order to determine which Fusarium species isolated from Colorado and Nebraska nursery soils and seedlings were pathogenic to conifer seedlings, pathogenicity tests using lodgepole pine seed and seedlings were conducted. Twelve isolates frequently observed on medium selective for Fusarium spp. and assumed to be Fusarium spp. were used in these tests. These isolates have been deposited at the Fusarium Research Center at Pennsylvania State University and identified.

MATERIALS AND METHODS

Lodgepole pine seed collected from the Grand Mesa, Uncompahgre, and Gunnison National Forest, Colorado at 3050 m elevation, seed lot PICO-04-09-486-100-83, was obtained from Mt. Sopris Nursery (U.S. Forest Service), Carbondale, Colorado. The lodgepole pine seed was cold-stratified and soaked in 3% hydrogen peroxide as recommended by Fuller and Hildebrand (1985).

Germinating Seeds

Pre- and post-emergence damping-off was observed with lodgepole pine seeds germinating at 21°C under diffuse light in a Scherer Environmental chamber, the optimum conditions for conifer germination (Tinus and McDonald, 1979). Twelve Fusarium isolates from the Colorado State Forest Service and U.S. Forest Service Bessey Nurseries were grown on potato dextrose agar (PDA) plates for about 10 days and then 6 mm diameter plugs of mycelium were cut with a sterilized cork borer. Each plug was placed on filter paper moistened with sterile water in a petri plate, and five seeds were arranged equidistant from the plug. Each Fusarium isolate was tested against 50 germinating seeds, and 50 seeds were germinated uninoculated as checks.

Germinating seeds were observed for four weeks, with dead seedlings removed weekly. After four weeks, ungerminated seeds were cut open to determine whether they were healthy (but dormant) or diseased. Diseased seeds and seedlings were plated on medium selective for Fusarium (Nash and Snyder, 1962) to reisolate the fungus.

Container Seedlings

Root rot was observed in lodgepole pine seedlings grown individually in 1:1 peat and vermiculite potting mix in Spencer LaMaire-type folding plastic containers in a Scherer Environmental chamber. After germination and thinning to one seedling per cell, each cell was inoculated with a plug of Fusarium sp. mycelium. Each of 11 Fusarium isolates was tested against 50 seedlings. Fifty additional seedlings in uninoculated potting mix were grown as checks. Seedlings were inoculated in groups of 16 or

less arranged randomly in an attempt to overcome effects due to differences in compaction of the potting mix.

Seedlings were observed for eight weeks after inoculation, during which time dead or dying seedlings were removed and examined for the cortical sloughing typical of Fusarium root rot. Roots of diseased seedlings were surface-sterilized and plated on selective medium to re-isolate the causal Fusarium species.

After the first four weeks at the optimum 24°C (16 hours) and watering twice weekly with nutrient solution (Tinus and McDonald, 1979), daytime temperature was changed to 30°C and watering to once weekly to stress the seedlings. This regime approximated midsummer conditions when Fusarium root rot usually becomes evident. Night temperatures were maintained at 18°C.

Since only a few seedlings died within the first eight weeks, each cell was reinoculated with a fresh Fusarium plug of the same isolate as before. Each plug was covered with a wad of sterile wet cotton to allow the fungus time to grow out of the plug before the agar dried up. After an additional four weeks, seedlings were watered once every two weeks, and after five weeks the temperature was kept at a constant 30°C to stress the seedlings.

The Fusarium isolates were ranked according to the incidence of disease in the lodgepole pine.

RESULTS AND DISCUSSION

Germinating Seeds

Table 1 gives the percentage of diseased seedlings or seeds which yielded in culture the same Fusarium isolate with which the filter paper plate was inoculated. Penicillium sp. was found to destroy about 4% of the seeds based on its incidence and effect in the check plates.

The range in disease incidence due to the Fusarium isolates was 8 to 76%. Isolates 85-29 and 85-19, F. moniliforme and F. oxysporum, were the most pathogenic, although different isolates of the same species differed somewhat in pathogenicity.

Table 1. Fusarium isolate and percentage of diseased lodgepole pine seeds germinating on filter paper which were infected with the same isolate with which plate was inoculated.

Isolate Number	Fusarium Species	Percentage Infected
85-17	equiseti	37
85-18	solani	30
85-19	oxysporum	70
85-20	semitectum	17
85-21	acuminatum	38
85-22	not Fusarium	13
85-23	acuminatum	46
85-24	not Fusarium	8
85-25	oxysporum	50
85-26	oxysporum	43
85-27	check	0
85-28	solani	30
85-29	moniliforme	76

Container Seedlings

When seedlings are stressed, those infected with pathogenic fungi tend to be the first to succumb. The heat and moisture stress resulted in many chlorotic and withered seedlings with high incidence of Fusarium infection, as presented in Table 2.

Eighteen percent of the check seedlings were infected with various Fusarium spp., contaminated with the isolates in the growth chamber. None of the unhealthy seedlings exhibited the cortical sloughing characteristic of Fusarium root rot. Since the average incidence of Fusarium infection was 24%, isolates 18, 19, and 29 were probably functioning as pathogens. The rest of the isolates probably entered the stressed seedlings as saprophytes or weak pathogens, not aggressively attacking root tissue. Other species may be involved in Fusarium root rot.

Simulation of field conditions in a growth chamber is difficult because many variables interact. Moisture stress was not constant between and within isolates.

Table 2. Fusarium isolate, percentage of unhealthy container seedlings, and percentage infected with the same isolate with which container was inoculated.

Isolate Number	Fusarium Species	Percentage Unhealthy	Percentage Infected by Isolate
85-17	equiseti	22	22
85-18	solani	35	35
85-19	oxysporum	35	35
85-20	semitectum	37	31
85-22	not Fusarium	27	27
85-23	acuminatum	14	12
85-24	not Fusarium	10	0
85-25	oxysporum	31	24
85-26	oxysporum	25	20
85-27	check	22	0
85-28	solani	27	27
85-29	moniliforme	35	35

The Fusarium isolates are ranked by pathogenicity in Table 3. All of these isolates except 85-24 (not Fusarium) grew on Komada's medium. Isolate 85-22 (not Fusarium) required six days at room temperature to exhibit growth on Komada's medium.

Table 3. Fusarium isolates ranked in order of decreasing pathogenicity.

Isolate Number	Fusarium Species
85-29	moniliforme Sheldon
85-19	oxysporum Schlect. emend. Snyd. & Hans.
85-25	oxysporum
85-18	solani (Mart.) Appel & Wollenw. emend. Snyd. & Hans.
85-23	acuminatum Ell. & Ev. sensu Gordon
85-26 *	oxysporum
85-21	acuminatum
85-17	equiseti (Corda) Sacc. sensu Gordon
85-28	solani
85-20 †	semitectum Berk. & Rav.
85-22	not Fusarium
85-24	not Fusarium

* Isolates above first line are highly pathogenic.
† Isolates below second line are non-pathogenic.

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